Concentration of Cryptosporidium, microsporidia and other water-borne pathogens by continuous separation channel centrifugation

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2001/221: received 1 August 2001, revised 10 September 2001 and accepted 23 October 2001

Aims: The aim of this study was to determine the effectiveness of continuous separation channel centrifugation for concentrating water-borne pathogens of various taxa and sizes.

Methods and Results: Cryptosporidium parvum oocysts, Giardia lamblia cysts, Encephalitozoon intestinalis spores and Escherichia coli were seeded into different water matrices at densities ranging from 5 to 10 000 organisms l⁻¹ and recovered using continuous separation channel centrifugation. All pathogens were enumerated on membrane filters using microscopy. Recovery efficiencies were usually > 90%. Oocyst recovery did not vary with source water turbidity or with centrifuge flow rate up to 250 ml min⁻¹. Based on excystation, this concentration method did not alter oocyst viability.

Conclusions: Continuous separation channel centrifugation is an effective means of concentrating water-borne pathogens.

Significance and Impact of the Study: Methods are needed for detecting pathogens in drinking water to ensure public health. The first step for any pathogen detection procedure is concentration. However, this step has been problematic because recovery efficiencies of conventional methods, like filtration, are often low and variable, which may lead to false negatives. Continuous separation channel centrifugation can simultaneously concentrate multiple pathogens as small as 1 μm with high and reproducible efficiency in a variety of water matrices.

INTRODUCTION

Cryptosporidium parvum is a coccidian gastrointestinal parasite that is ubiquitous in surface waters (LeChevallier et al. 1991a; Rose et al. 1991; Lisle and Rose 1995). It has been responsible for a number of disease outbreaks related to drinking water (Craun et al. 1998). Giardia lamblia is another familiar water-borne parasite and lately, there has been concern over the water-borne transmission potential of other eukaryotic enteric pathogens such as Toxoplasma gondii, Cyclospora sp. and microsporidia (Rabold et al. 1994; Bowie et al. 1997; Cotte et al. 1999). To protect public health, the approach taken in the US and UK is to monitor drinking water and source waters for Cryptosporidium oocysts and Giardia cysts, and methods are currently being developed to detect microsporidia spores.

The first step in any detection method for water-borne pathogens is concentration. Concentration is necessary because the ambient densities of pathogens in water are usually less than the limit of detection, and concentrating over a period of time may compensate for spatial and temporal variations in pathogen occurrence. The detection methods for many water-borne pathogens are state-of-the-art, like polymerase chain reaction (PCR) and flow cytometry. However, some concentration methods can be remarkably ineffective, increasing the likelihood of false negatives. For example, the filter cartridge concentration method used in a national occurrence study of Cryptosporidium in the US was reported to have a recovery efficiency of only 3% (Clancy et al. 1994). The objective of this study was to determine the effectiveness of continuous separation channel centrifugation for concentrating Cryptosporidium and several other water-borne pathogens of different sizes.
MATERIALS AND METHODS

Centrifuges

Experiments were conducted with three blood cell separators, an IBM model 2997 (now obsolete), a COBE Spectra (Lakewood, CO, USA) and an Amicus Separator (Baxter Healthcare Corp., Round Lake, IL, USA). The separation channel for the IBM and COBE models is akin to a hollow belt (Fig. 1). The Amicus uses a flexible bag partitioned into a serpentine flow path. Regardless of the channel design, the centrifuges operate under the same separation principle. An inner wall separates the channel inlet from the outlet so that water pumped into one end must traverse the entire channel length before exiting. The water flow path is tangential with respect to the rotational axis of the rotor (not shown) so that centrifugal force is directed perpendicular to the outer channel wall. Particulates, including micro-organisms, are retained in the channel by centrifugal force (Fig. 2) as long as the settling time of the particulate is less than the travel time of the water in the channel. The process is continuous and there is no filtration involved.

Centrifugation procedure

The blood cell separators were operated as simple continuous centrifuges; unnecessary blood separation components were disabled or by-passed. The separation channel was primed with sterile 0.001% Tween 80 (Sigma). Water was pumped through a single input line into the separation channel using a peristaltic pump (model 7553–20, Cole Parmer, Vernon Hills, IL, USA) and the supernatant fluid left the channel via a single output line. Rotor speed was set at maximum for all experiments, resulting in a relative centrifugal force (RCF) at the channel width midpoint of approximately 900 g.

After centrifugation was complete, the channel was removed from the rotor and the concentrate drained into a sterile beaker. The channel was cut open, filled with 20–30 ml 0.01% Tween 80, clamped, and shaken vigorously with a laboratory vortex mixer. Several rinses were performed and combined with the concentrate. The final volume was 200–250 ml for the IBM and COBE Spectra centrifuges and 50–70 ml for the Amicus centrifuge. The separation channels are sterile, disposable and used only once.

Seeded pathogens

*Cryptosporidium parvum* oocysts (Iowa isolate) and *Giardia lamblia* cysts were purchased live and purified from PRL Dynagenics (Neosho, MO, USA) for the recovery experiments. (Oo)cysts were diluted with distilled water to make working stock solutions of between $5 \times 10^3$ and $1.5 \times 10^4$ oocysts ml$^{-1}$ and $2.2 \times 10^4$ cysts ml$^{-1}$. For the viability experiment, oocysts (Iowa isolate) were obtained from the Sterling Parasitology Laboratory (University of Arizona-Tucson, USA).

Spores of the microsporidian *Encephalitozoon intestinalis* were purchased from two sources: live from Waterborne Inc. (New Orleans, LA, USA), and fixed in 3% formalin from PRL Dynagenics. Spores from the latter source were cleaned by density gradient centrifugation with histopaque before use. Densities of microsporidia working stock solutions were between $1 \times 10^4$ ml$^{-1}$ and $5 \times 10^5$ ml$^{-1}$.

*Escherichia coli* (ATCC 25922) was grown overnight on sheep blood agar at 35°C and diluted in sterile saline to working stock concentrations of $1 \times 10^9$ ml$^{-1}$ before seeding.

### Fig. 1
Schematic representation of the IBM 2997 and COBE Spectra separation channel.

### Fig. 2
Separation channel of the IBM 2997 filled with concentrate from pond water sample. Channel diameter = 27 cm. The arrow indicates the location of concentrated sediment and micro-organisms near the channel inlet.
Densities of all working stock solutions were determined on the same day that samples were seeded, using the same enumeration method as for the recovered pathogens.

Pathogen enumeration

*Cryptosporidium* and *Giardia* were enumerated by vacuum filtering (≤ 100 mmHg) the centrifuge concentrate through a 1-0 µm absolute pore diameter, 25 mm, pre-blackened, polycarbonate membrane (Poretics, Livermore, CA, USA) underlain with a polyester drain disc (Poretics), and held in a polycarbonate locking filter holder (Pall Gelman Laboratories, Ann Arbor, MI, USA). For oocyst recovery from simple water matrices, 53–100% of the concentrate volume was filtered. For the other *Cryptosporidium* and *Giardia* recovery experiments, three to eight 0.5–5.0 ml aliquots were filtered. Membranes were saturated with 70 µl of a 1:2 dilution of FITC-labelled anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies (Merifluor *Cryptosporidium*/*Giardia* Detection Kit, Cincinnati, OH, USA) and transferred to a glass slide. In simple water matrices when the seed density was ≤ 20 organisms l⁻¹, the entire membrane was counted, otherwise > 100 fields were counted. For complex water matrices, the entire filter was counted. Magnification was 200×; presumed oocysts and cysts were confirmed at 400× based on external morphology.

For *E. coli* and microsporidia, the entire centrifuge concentrate was filtered through a 0.22 µm absolute pore diameter polycarbonate membrane. In the *E. coli* experiments, 500 ml supernatant fluid were also filtered and counted. The *E. coli* and microsporidia used in the saline recovery experiments were stained with 100 µl DAPI (10 µg ml⁻¹). The microsporidia used in the reagent-grade water experiments were labelled with 100 µl 1:20 dilution of anti-Microsporidium-FITC antibody (Microspor-Fa, Waterborne Inc.) applied to the membrane, incubated for 30 min at room temperature, rinsed twice with 5 ml phosphate-buffered saline (PBS) and mounted on a glass slide with 2% DABCO. The saline and reagent-grade water negative controls showed no DAPI-stained or FITC-labelled micro-organisms or particles. At microsporidia seed densities of 10 l⁻¹, the entire filter was counted; at the other microsporidia and *E. coli* seed densities, generally > 400 organisms were counted from 50–1200 fields. Magnification was 1000×. Counts of all seeded pathogens were performed with a Nikon Optiphot epifluorescent microscope.

Recovery experiments

Recovery from simple water matrices. Simple water matrices included sterile PBS, sterile isotonic saline, reagent-grade water and tap water. *Cryptosporidium* was killed with formalin (10% final concentration) in a 100 ml aliquot of the test water before seeding. *Escherichia coli* was seeded live. *Encephalitozoon intestinalis* was seeded live for recovery from reagent-grade water, and formalin-fixed (3%) for the saline experiments.

Recovery from complex water matrices. Surface water from two local ponds was used to test recovery of *Cryptosporidium* and *Giardia* from complex water matrices. Both organisms were killed with 10% formalin before seeding. An unseeded water sample of a volume equal to the seeded sample was centrifuged to quantify indigenous oocysts and cysts in the ponds. *Giardia* cysts were detected on one occasion; oocysts were never detected. Turbidity was measured with a fluorescence spectrophotometer (Hitachi Model F-4500, San Jose, CA, USA).

Effect of centrifuge flow rate on oocyst recovery. Two sets of experiments were performed, one with the COBE Spectra (seed density = 1000 oocysts l⁻¹) and the other with the Amicus (seed density = 100 oocysts l⁻¹). In both sets, oocysts were seeded into 10 L pond water and recovered using four centrifuge flow rates: 70, 150, 250 and 500 ml min⁻¹.

Effect of turbidity on oocyst recovery. Oocysts were seeded at a density of 100 l⁻¹ into 7.5 L pond water or 10 L utility source water. Pond water turbidity was experimentally manipulated by augmenting test volumes with pond seston concentrated by bulk centrifugation (10 000 g for 15 min). Source waters were from seven municipal utilities, selected for their naturally-occurring turbidity levels. Source water (20 L) was shipped overnight and used in recovery experiments the next day. Utility personnel measured turbidity at the time of collection. A 10 L unseeded control was centrifuged to account for indigenous oocysts.

Calculating recovery efficiency. Percent recovery was calculated as the number of pathogens recovered in the centrifuge concentrate divided by the total number seeded multiplied by 100. To correct for indigenous (oo)cysts, the number in surface water controls was subtracted from the number recovered. The denominator for *E. coli* recovery efficiency was the total number of bacteria present at the end of centrifugation to account for any increase due to cell division. Percent recovery was calculated as the number of *E. coli* in the concentrate divided by the sum in the concentrate and supernatant fluid.

Effect of centrifugation on *Cryptosporidium* viability

Twenty-five litres of pond water (n = 3) or sterile saline (n = 3) were seeded with live oocysts to a final concentration of 80 000 oocysts l⁻¹ and centrifuged at 900 g and
a flow rate of 150 ml min\(^{-1}\) with the COBE Spectra. Control oocysts were seeded into a 1 litre aliquot of the water matrix and incubated on the bench at room temperature parallel to the time the 25 L sample was being concentrated. Viability was evaluated by excystation following the protocol of M. Marshall, University of Arizona. Oocysts in a 2 ml aliquot of the centrifuge concentrate or control were spun at 15 000 g for 1 min and washed twice in Hank’s balanced salt solution. The final pellet was resuspended in 500 ml excystation media (0.5% trypsin and 1.5% sodium taurocholate) and incubated for 4 h at 37°C. Oocysts were examined by phase microscopy. Excystation rate was calculated as the number of ‘ghosts’ divided by the sum of intact oocysts and ‘ghosts.’

**Statistical analyses**

Data were examined for normality and heteroscedascity, and transformations or non-parametric tests were applied as required. All reported means are arithmetic. The relationship between organism type and density and recovery efficiency in simple and complex water matrices was tested by two way analysis of variance. For recovery of *Cryptosporidium* in simple matrices, data were transformed by the Box-Cox method, and the effect of oocyst density on recovery was assessed by linear regression. Correlations were determined by the Pearson correlation coefficient. All statistical analyses were performed using SAS version 6.12 (SAS 1990).

**RESULTS**

**Recovery from simple water matrices**

*Cryptosporidium parvum*, *E. coli* and microsporidia were recovered from 2–20 L volumes of PBS, saline, reagent-grade water and tap water at efficiencies typically > 90% (Table 1). At a centrifuge flow rate of 70 ml min\(^{-1}\) and seed densities of 100 or 1000 organisms l\(^{-1}\), recovery efficiency varied with organism type \( (P < 0.001) \) and density \( (P = 0.013) \). Recovery of *Cryptosporidium* was lower than that of *E. coli* \( (P = 0.001) \) and microsporidia \( (P < 0.001) \), but *E. coli* and microsporidia recovery efficiencies were similar \( (P = 0.745) \). *Cryptosporidium* recovery showed a decreasing trend with increasing oocyst density \( (P = 0.016) \). At a flow rate of 150 ml min\(^{-1}\), nearly all microsporidia spores seeded into reagent-grade water and all *Cryptosporidium* oocysts seeded into tap water were recovered (Table 1).

**Recovery from complex water matrices**

Recovery of *Cryptosporidium* and *Giardia* seeded into naturally-occurring water from two local ponds was usually ≥ 95% (Table 2). The centrifuge flow rate in

<table>
<thead>
<tr>
<th>Organism</th>
<th>Water matrix</th>
<th>Organisms l(^{-1})</th>
<th>Water volume centrifuged (L)</th>
<th>Centrifuge flow rate (ml min(^{-1}))</th>
<th>Number independent trials</th>
<th>Mean recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variation (%)</th>
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<tr>
<td><em>Cryptosporidium</em></td>
<td>PBS*</td>
<td>5</td>
<td>5</td>
<td>70</td>
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<td>99</td>
<td>16.7</td>
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<td>70</td>
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<td>98</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td>92</td>
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<td>5.4</td>
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<td>Microsporidia</td>
<td>Reagent-grade water†</td>
<td>10</td>
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<td>150</td>
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<td><em>Cryptosporidium</em></td>
<td>Tap water*</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>7</td>
<td>104</td>
<td>16.0</td>
<td>15.4</td>
</tr>
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</table>

*Centrifuged with IBM 2997.
†Centrifuged with COBE Spectra.
these experiments was 150 ml min⁻¹. Recovery efficiencies did not differ significantly by organism type \((P = 0.502)\) or density \((P = 0.111)\). For Cryptosporidium alone, recovery at 20 oocysts L⁻¹ was significantly lower than at densities of 100 oocysts L⁻¹ and 1000 oocysts L⁻¹ \((P = 0.035)\). Cryptosporidium oocysts seeded into 100 L volumes of pond water were recovered at an efficiency similar to experiments using smaller volumes.

### Effect of centrifugation on Cryptosporidium viability

Viability of Cryptosporidium oocysts was not altered by continuous separation channel centrifugation. In both saline and pond water (1.3 NTU), the excystation rate was ≥94%, and the rate was nearly identical between centrifuged and control oocysts (Table 3).

### Table 2 Recovery of Cryptosporidium and Giardia from local pond water using continuous separation channel centrifugation. Centrifuge flow rate = 150 ml min⁻¹

<table>
<thead>
<tr>
<th>Organism</th>
<th>Water volume centrifuged (L)</th>
<th>Turbidity (NTU)</th>
<th>Number independent trials</th>
<th>Mean recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>20 25*</td>
<td>8.3</td>
<td>3</td>
<td>78</td>
<td>17.9</td>
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<td></td>
<td>100 5†</td>
<td>10.1</td>
<td>1</td>
<td>92</td>
<td>—</td>
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<tr>
<td></td>
<td>100 7†</td>
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<td>109</td>
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<tr>
<td></td>
<td>100 100†</td>
<td>2.8</td>
<td>3</td>
<td>95</td>
<td>9.7</td>
<td>10.2</td>
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<td>8.3</td>
<td>4</td>
<td>101</td>
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</tr>
<tr>
<td></td>
<td>1000 10*</td>
<td>8.3</td>
<td>3</td>
<td>97</td>
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<td>9.9</td>
</tr>
<tr>
<td>Giardia</td>
<td>20 30*</td>
<td>8.3</td>
<td>3</td>
<td>97</td>
<td>10.5</td>
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<td>104</td>
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<td>5.5</td>
</tr>
</tbody>
</table>

*Centrifuged with IBM 2997.
$†$Centrifuged with COBE Spectra.

For experiments with the COBE Spectra (8.3 nephelometric turbidity units (NTU) pond water), mean recovery was ≥90% at flow rates of 70 and 150 ml min⁻¹, but decreased substantially at higher flow rates (Fig. 3). For the Amicus Separator (2 NTU pond water), recovery was still near 100% at 250 ml min⁻¹ and only decreased to 72% at 500 ml min⁻¹.

### Effect of centrifuge flow rate on oocyst recovery

Oocyst recovery did not vary with turbidity levels between 1 and 45 NTU \((r = 0.2, P = 0.52)\) (Fig. 4). Recovery of 100 Cryptosporidium oocysts L⁻¹ seeded into utility source waters ranged from 80% (Lake Michigan, Kenosha, WI) to 106% (Iowa River, Iowa City, IA). The variability of the oocyst counts was associated with turbidity \((r = 0.85, P < 0.001)\), as evidenced by the width of the standard deviation bars in Fig. 4.

### Effect of centrifugation on Cryptosporidium viability

Viability of Cryptosporidium oocysts was not altered by continuous separation channel centrifugation. In both saline and pond water (1.3 NTU), the excystation rate was ≥94%, and the rate was nearly identical between centrifuged and control oocysts (Table 3).

![Fig. 3](image-url) Effect of centrifugation flow rate on oocyst recovery using the COBE Spectra (■) and Amicus Separator (○). Error bars indicate ± 1 S.D. of three to five independent trials at each flow rate.
DISCUSSION

This study has demonstrated that continuous separation channel centrifugation is an effective means of concentrating water-borne pathogens of various taxa and sizes. Cryptosporidium parvum oocysts (5 μm), Giardia lamblia cysts (10 μm), E. coli (1 μm) and Encephalitozoon intestinalis spores (1 μm) were routinely recovered with 90% or greater efficiency. High recovery efficiency coupled with sensitive and specific detection methods translates to improved accuracy of pathogen monitoring and reduced risk of water-borne disease.

The Cryptosporidium and Giardia recovery efficiencies reported here for continuous separation channel centrifugation were generally higher than alternative methods. The polypropylene yarn cartridge filtration method, used in the US EPA Information Collection Rule, has reported oocyst recovery efficiencies of 25-3 to 42% (LeChevallier et al. 1991b), 1:3 to 5:5% (Clancy et al. 1994), 8% (Nieminski et al. 1995), and as low as 0% (Fricker 1995); these same studies showed that the method does not recover Giardia cysts much better. Using a wound fibreglass cartridge filter, Kaucner and Stinear (1998) reported Cryptosporidium and Giardia recovery efficiencies of 54% and 74%, respectively. Filtration through large diameter polycarbonate membranes recovered oocysts from river water at efficiencies of 5 to 22% (Ongerth and Stibbs 1987), and from tap water with efficiencies of 25-5% (Dawson et al. 1993) and near 100% (Clancy et al. 1997). With cellulose acetate membranes, oocysts were captured with efficiencies of 70-5% (Aldom and Chagla 1995) and 78-8% (Graczek et al. 1997). Recovery efficiencies of membrane filters have been reported to vary with type of filter matrix and pore size (Shepherd and Chagla 1995) and 78% (Graczek et al. 1997). Recovery of Cryptosporidium oocysts, and oocyst recovery efficiencies as high as 79% have been experienced VFF operators reported oocyst recovery from tap water as high as 103% (Riley et al. 1998). Calcium carbonate precipitation is a filterless concentration method and oocyst recovery efficiencies as high as 79% have been reported (Vesey et al. 1993); however, there is evidence that the high pH conditions impair oocyst viability (Campbell et al. 1994). All these methods have their advantages and disadvantages and may be the best method for a particular set of environmental conditions or monitoring objectives.

Currently, the most commonly employed methods for concentrating Cryptosporidium oocysts are the Envirochek filter (Pall Gelman Laboratories, Ann Arbor, MI, USA) and Filta-Max filter (IDEXX Laboratories, Westbrook, ME, USA). The former is a capsule-enclosed polyethersulphone membrane specifically designed to capture oocysts and cysts. Source water recovery efficiencies were 51% (Clancy et al. 1997) and 63% (Matheson et al. 1998) for Cryptosporidium oocysts, and 85% (Clancy et al. 1997) and 75% (Matheson et al. 1998) for Giardia cysts. With finished water, the Envirochek capsule sometimes recovered all seeded oocysts and cysts (Clancy et al. 1997). The Filta-Max filter is composed of compressed foam rings that, when decompressed, release entrapped particles. The reported recovery efficiency for this filter for oocysts seeded into 10 L volumes of river water is 89% (Sartory et al. 1998). Despite the plethora of data on method recovery efficiencies, it is

Table 3 Effect of continuous separation channel centrifugation (COBE Spectra) on Cryptosporidium oocyst viability

<table>
<thead>
<tr>
<th>Water type</th>
<th>Number independent trials</th>
<th>Percent excysted mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Centrifuged</td>
</tr>
<tr>
<td>Saline</td>
<td>3</td>
<td>94.4 ± 2.1</td>
</tr>
<tr>
<td>Pond water</td>
<td>3</td>
<td>96.3 ± 0.9</td>
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difficult to compare the performance of just the concentration component of an entire pathogen detection method when studies differ in their choice of the purification and enumeration method components (Lindquist et al. 1999). Experimental conditions such as turbidity, seeding density, sample volume and the number of replicates also differ among studies.

Two recent studies have evaluated continuous centrifugation as a means of concentrating Cryptosporidium and Giardia. Zuckerman et al. (1999) modified a Haemonetics blood cell separator (Haemonetics Co., Braintree, MA, USA) for portable use in the field. Recovery experiments were performed at a flow rate of 1.4 litres min–1 and seed densities of 26 000 oocysts l–1 or 11 000 cysts l–1 in 45 litres of water. Average recovery efficiencies from tap water and river water were 87.7 and 56.7%, respectively, for Giardia, and 55.3 and 26.8%, respectively, for Cryptosporidium. The highest recovery efficiency of oocysts in tap water was 94%.

Swales and Wright (2000) used an MSE model 300 basket centrifuge (no longer manufactured) for concentrating Cryptosporidium from 100 L water volumes. At a flow rate of 750 ml min–1 and 2900 g, and using a detection method similar to the present study (i.e. fluorescence microscopy of oocysts on membranes without additional purification steps after concentration), the average recovery efficiency of oocysts seeded into tap water at densities of 100, 50, 14 and 5 oocysts l–1 was 70.3, 51.0, 37.8 and 28.6%, respectively. Both these studies used bowl-type continuous centrifuges, which achieve particle concentration under a different configuration than the separation channel centrifuges used in this study.

Besides high recovery efficiency, continuous separation channel centrifugation offers the following additional advantages for concentrating water-borne pathogens. (i) Multiple bacterial and protozoan pathogens can be concentrated simultaneously, reducing the need for multiple concentration methods. How efficiently taxa of different sizes and specific gravity are simultaneously concentrated will depend on centrifuge flow rate and RCF, but these parameters can be adjusted to optimize recovery efficiency. The high recovery efficiencies for microsporidia reported in this study are notable because these are emerging water-borne pathogens for which there are no standard methods for concentration. (ii) Concentration is effective with a variety of water matrices. In this study, oocyst recovery was not correlated with turbidity levels ranging from 1 to 45 NTU. (iii) The performance of the centrifuge is highly reproducible. The highest coefficient of variation among independent trials was 35%, the lowest was 0.7% and usually, it did not exceed 20%. (iv) Concentration occurs without a filter, so clogging and breakthrough are avoided and there is no filter matrix or eluent that could later interfere with the chosen detection method. (v) The centrifugation process operates under low g forces, preserving pathogen integrity and, consequently, it is compatible with a greater number of detection methods. In this study, continuous separation channel centrifugation did not alter oocyst excystation. There is also evidence that continuous separation channel centrifugation does not alter the epitopes which provide the basis for several immunofluorescence detection methods (Hoffman et al. 2000). (vi) Lastly, concentration may be performed continuously, providing time-integrated sampling.

The primary limitation of continuous separation channel centrifugation is the slow flow rate. With the COBE Spectra, recovery efficiency of Cryptosporidium oocysts decreased substantially at flow rates > 150 ml min–1. However, with the Amicus Separator, oocysts recovery was still 99% at 250 ml min–1 and decreased to only 72% at 500 ml min–1. The recovery efficiencies probably differed between centrifuges because of the design of their separation channels. Compared with the COBE channel, the Amicus channel has a greater surface area, and surface area is directly related to the centrifugal sedimentation capacity of a chamber (Brown 1989). A separation channel that yields high pathogen recovery efficiencies at a flow rate of 1 litre min–1 is currently being designed.

Another limitation is that under extremely turbid conditions and large sample volumes, the channel may eventually become filled with particulates, halting further concentration. Future tests should include highly turbid water (> 100 NTU). Lastly, designed as blood cell separators, continuous separation channel centrifuges are too expensive and complicated to be a practical method of concentrating water-borne pathogens. The objective of this study was to test the principle of continuous separation channel centrifugation rather than a specific device. A prototype centrifuge designed specifically for concentrating water-borne pathogens is currently being developed. It is anticipated that the cost per sample would be in the order of tens of US dollars, not including labour or the initial expense of the centrifuge.

The principle of continuous separation channel centrifugation is simple and provides several advantages over filter-based concentration methods. Compared with state-of-the-art pathogen detection methods, concentration methods are prosaic. However, in this time of emerging pathogens and recurring water-borne diseases, all procedural steps for pathogen monitoring, even the most basic, should be improved and alternatives considered.

ACKNOWLEDGEMENTS

The authors thank Ann Cali for confirming the identity of E. intestinalis, Beth Frankowiak and Phil Bertz for laboratory assistance, Richard Berg for performing the statistical analyses, Lynn Ivacic for graphic arts support and Alice

© 2002 The Society for Applied Microbiology, Journal of Applied Microbiology, 92, 649–656
Stargardt for manuscript preparation. Baxter Healthcare Corporation kindly provided the Amicus centrifuge and disposables. This study was funded by a Marshfield Clinic internal grant.

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